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SHORT REPORT

Fluorescent cDNA microarray hybridization reveals complexity and heterogeneity of cellular genotoxic stress responses

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The fate of cells exposed to ionizing radiation (IR) may depend greatly on changes in gene expression, so that an improved view of gene induction profiles is important for understanding mechanisms of checkpoint control, repair and cell death following such exposures. We have used a quantitative fluorescent cDNA microarray hybridization approach to identify genes regulated in response to γ-irradiation in the p53 wild-type ML-1 human myeloid cell line. Hybridization of the array to fluorescently-labeled RNA from treated and untreated cells was followed by computer analysis to derive relative changes in expression levels of the genes present in the array, which agreed well with actual quantitative changes in expression. Forty-eight sequences, 30 not previously identified as IR-responsive, were significantly regulated by IR. Induction by IR and other stresses of a subset of these genes, including the previously characterized *CIP1/WAF1*, *MDM2* and *BAX* genes, as well as nine genes not previously reported to be IR-responsive, was examined in a panel of 12 human cell lines. Responses varied widely in cell lines with different tissues of origin and different genetic backgrounds, highlighting the importance of cellular context to genotoxic stress responses. Two of the newly identified IR-responsive genes, *FRA-1* and *ATF3*, showed a p53-associated component to their IR-induction, and this was confirmed both in isogenic human cell lines and in mouse thymus. The majority of the IR-responsive genes, however, showed no indication of p53-dependent regulation, representing a potentially important class of stress-responsive genes in leukemic cells.

Keywords: cDNA microarray; ionizing radiation; leukemia; p53; genotoxic stress

Genotoxic stresses, such as ionizing radiation (IR), can elicit a wide variety of cellular responses, from cell-cycle arrest to mutation, transformation, or cell death. On the molecular level, early responses to IR include activation of proteins such as p53 and NFκB, changes in protein localization, engagement of signal cascades and transcriptional induction of specific genes such as *CIP1/WAF1* and *MDM2*. Many factors, both cell-type and stress-specific, can interact to determine the final outcome for the cell. IR has proved a useful probe for the study of basic processes such as cell-cycle regulation, apoptotic pathways, and DNA metabolism and the identification of new IR-response genes may also provide novel targets for future experimental approaches in radiotherapy.

The human myeloid cell line ML-1 was selected for this study based on our previous experience with stress gene induction in many different cell lines. Various factors contribute to the IR-responsiveness of cell lines. For instance, the p53 wild-type status of ML-1 allowed the detection of many genes, such as *CIP1/WAF1, GADD45* and *MDM2*, which require p53 function for optimal IR-induction. Importantly, this cell line contains endogenous wild-type p53, so the results obtained represent cellular responses with physiological levels of p53, rather than the unnatural and often highly overexpressed levels resulting from artificially engineered systems. In addition, the fact that ML-1 is a myeloid cell line, prone to undergo rapid apoptosis following genotoxic stress, also results in the induction of genes specifically associated with this process, such as *BAX, MCL1, GADD34* and *BCL-X_L* (Zhan *et al*., 1994b, 1997). The genes we knew to be IR-regulated in ML-1 prior to this study gave us a wide range of target responses we could expect to detect on the microarray, from >50-fold induction for *CIP1/WAF1* to approximately tenfold reduction for c-MYC.

Since the initial development of cDNA microarray hybridization (Schena *et al*., 1995), there has been considerable interest in this rapidly emerging technology. The ability to compare relative levels of thousands of mRNA transcripts simultaneously in a single hybridization has the potential to contribute greatly to our understanding of many different fields. Development of a quantitative approach for the measurement of relative changes in gene expression would offer the added advantage of expanding this approach beyond simple pair-wise comparisons. Demonstrated applications have included monitoring of differential gene expression in wild-type and transgenic plants (Schena *et al*., 1995) and of a human melanoma cell line and its non-tumorigenic variant (DeRisi *et al*., 1996), expression of heat-shock genes in a human cell line (Schena *et al*., 1996), metabolic shift in yeast (Lashkari *et al*., 1997), and the identification of genes related to inflammatory disease (Heller *et al*., 1997).

Transcriptional stress-response is an extremely complex area, with the response of many genes dependent on intermeshing signal transduction activ-

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ities, cell type specific factors and genetic background. This can make it difficult to assess the importance of the response of a single gene on the basis of data from one or a few cell lines or individuals. The application of a high-throughput screening method, such as cDNA microarray hybridization, may be necessary to develop a clearer picture of stress-responsive pathways. We have extended the application of fluorescent cDNA microarray hybridization to the study of a complex stress-responsive system in a non-engineered human cell line.

The microarrays used in this study included a general sampling of human genes (622 ESTs) plus another set of genes (616 ESTs) which were chosen on the basis of their known roles in cancer or lymphoid biology. A panel of housekeeping genes and other internal controls was also included on the array. All ESTs were selected from the UniGene database with the assistance of G Schuler and M Boguski. A representative quadrant of a hybridized microarray is shown in Figure 1. Induced transcripts hybridize with more of the probe from the IR-treated sample (labeled in red), resulting in red spots, such as that seen at the *CIP1/WAF1* target indicated in Figure 1. A transcript down-regulated by IR, such as c-MYC, would similarly produce a green spot (Figure 1). Intermediate induction ratios result in a gradation of color, such as *MBP-1* in Figure 1.

Among the transcripts significantly changed by radiation treatment (Table 1) were a number of genes previously known to be radiation inducible, as well as many genes not previously reported as ionizing-radiation regulated. Table 1 also gives the mean intensities of hybridization to the unirradiated control on the microarray. This measure has previously been shown to correlate roughly with transcript abundance (Schena *et al*., 1995, 1996), and demonstrates identification of IR-modulated genes over three orders of magnitude of basal expression. It should be noted that many of the stress regulated transcripts identified in Table 1 are known to be expressed at very low levels in ML-1 cells, consistent with their relative hybridization intensities on the array. For example, *GADD45* and *CIP1/WAF1* represent approximately $1/10^5$ transcripts in unirradiated cells.

A subset of the IR-responsive genes indicated by the microarray with a range of relative ratios were chosen for further study. Probes were obtained from the same plasmids used as targets on the array and γ-ray induction of these genes was confirmed in independent experiments by Northern blot hybridization (Figure 2). Estimates of induction or repression as measured by the microarray were compared to quantitative hybridization with single labeled probes. As indicated in Table 1, estimated expression varied by less than twofold for many transcripts. As will be discussed in more detail elsewhere (Bittner *et al*., in preparation), induction in some cases diverged from that measured by microarray hybridization in an apparently EST specific manner. However, it should be noted that all tested sequences that were identified on the microarray as induced showed an appreciable (>2-fold) induction by the quantitative hybridization approach, with the exception of *MRC-OX*, which showed only 1.5-fold induction. In the case of genes showing less than 2.4-fold induction by the microarray, useful data may still be obtainable. For example, *MCL-1* showed 1.9-fold induction by the microarray and 2.5-fold induction by quantitative hybridization. This gene has previously been shown

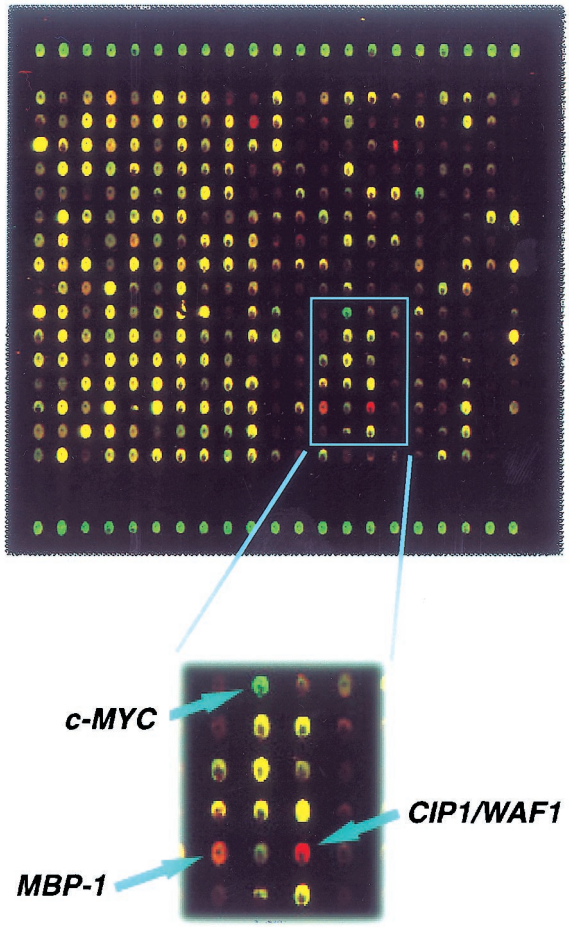

Figure 1 One quadrant of the microarray hybridized to RNA from untreated ML-1 cells (green fluorochrome) and ML-1 cells 4 h after treatment with 20 Gy^{137} Cs γ-rays (red fluorochrome). Targets appearing as yellow spots have equal representation of both fluorochromes and indicate no change in expression by the IR treatment. Red spots are targets increased by the treatment, and green targets are decreased. The horizontal row of green dots, 'landing lights', at the top and bottom are DNA labeled prior to printing on the array and serve as orientation markers for the computerized scanner. Representative target spots are identified in the enlarged segment. EST targets were prepared by PCR amplification and arrayed on poly-L-lysine coated glass slides by high speed robotic printing as previously described (DeRisi *et al*., 1996). A complex cDNA probe was prepared from whole-cell RNA by a single round of reverse transcription in the presence of fluorescent dNTP (Cy3 dUTP or Cy5 dUTP, Amersham). Probes were hybridized to the slides for 16 h in $3\times$SSC at 65°C in the presence of blockers. Hybridized slides were washed at room temperature in $0.5\times$SSC, 0.01% SDS, then in $0.06\times$SSC. The two fluorescent intensities were scanned separately using a laser confocal microscope, and the DeArray program was then used to identify target sites by image segmentation, calibrate relative ratios, and to develop confidence intervals for testing the significance of the ratios obtained (Chen *et al*., 1997). Local background was calculated for each target location. A normalization factor was estimated from a set of 88 internal control targets (DeRisi *et al*., 1996) with a theoretical ratio of 1.0, and the confidence interval for the array was estimated from the variance of these 88 control ratios from the expected value of 1.0. The ratios for all the targets on the array were then calibrated using the normalization factor, and ratios outside the 99% confidence interval (<0.54 or >2.37) were determined to be significantly changed by the radiation treatment

Table 1 Stress gene responses in γ-irradiated human myeloid cells

Transcript	*Image ID*	*Microarray*[a]	*Mean Green Intensity*[b]
CIP1/WAF1	268652	42.0[c]	1121
ATF3	428248	11.3[c]	784
FAS	151767	9.5	1352
IAP-1	129632	9.3[d]	132
RELB	52681	7.2[d]	1027
CYCLIN-I	512391	6.9	974
RAB	52530	6.4	1688
GADD45	310141	5.8[d]	2230
FRA1	110503	4.8[c]	672
IL-8	328692	4.8	5307
CSF-1	124554	4.7	356
BCL3	236422	4.6[c]	584
MIP1α	153355	4.3	475
c-FOS	26474	4.2	1314
JUN-B	309477	4.2	1892
PC-1	52753	3.4[c]	343
CDC5	280376	3.4	1259
MRC OX-2	51363	3.4[d]	290
ERR1	470602	3.2	490
MDM2	147075	3.2[d]	328
Immunoglobulin J chain	161023	3.1	331
OX40 ligand	35326	3.1	310
DNA ligase III	470062	3.0	636
cytochrome p450 4A	120466	3.0	218
MEK1	486074	2.9	1885
TPK receptor UFO	112500	2.8	285
Retinoic acid gamma-1	471252	2.8	288
HPTP alpha	487130	2.8	282
MBP-1	291503	2.8[c]	2065
SSAT	41452	2.7[c]	4160
BAK	[e]	2.7[c]	341
MBP-2	23464	2.5	462
iL-TMP	243143	2.5[d]	383
MIP1beta	205633	2.5	352
N-RAS	284031	2.5	341
nucleotide binding protein mRNA	21420	2.5	394
CAP-R	48677	2.4	577
BCL-X_L	[e]	2.3[c]	313
ch-TOG	43060	0.53	9209
ERK	487417	0.51	9323
CDC2	346534	0.47	19501
SATB1	233194	0.46[c]	30364
SPI-B	295093	0.45	3747
ERF-1	48085	0.44	6935
MADP2 homolog	26541	0.43	20543
neuron-specific protein gene	28089	0.38	8098
TOPO II	248032	0.34[c]	37173
CDK RS2	359119	0.27	13935
RCH-1	28116	0.23[c]	24932
c-MYC	51699	0.12[c]	17913

[a]Ratios of relative induction by γ-rays compared to basal levels in ML-1 cells. See also http://rex.nci.gov/RESEARCH/basic/lbc/fornace/htm. [b]Fluorescence intensity of untreated control on the microarray. [c]Microarray measurement confirmed by quantitative dot blot hybridization where expression varied by less than twofold. (Bittner *et al*., in preparation). [d]Quantitation varied by more than twofold (Bittner *et al*., in preparation). [e]Inserts for *BAK* (Chittenden *et al*., 1995) and *BCL-X_L* (Boise *et al*., 1993) from clones other than Image Consortium ESTs

to be IR-responsive in the ML-1 cell line (Zhan *et al*., 1997). The full listing of sequences used on this microarray and their relative ratios can be accessed via the website at http://rex.nci.nih.gov/RESEARCH/basic/lbc/fornace.htm.

The timecourse of induction for nine of these genes was examined in ML-1 cells. The response over time of Rag cohort 1 (*RCH1*) (Cuomo *et al*., 1994), a newly recognized IR-down-regulated gene, was very similar to the response of *TOPOII* (data not shown). Both

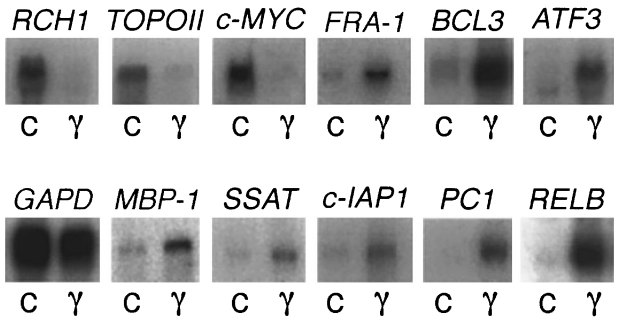

Figure 2 Representative Northern blot analysis confirming genes showing altered expression by the microarray following IR treatment. C: untreated control, γ: 4 h after γ-irradiation. Molecular weights of hybridizing bands were consistent with published transcript sizes. ATF3 hybridized to two different sized bands, corresponding to the alternately spliced forms previously reported (Chen *et al*., 1994a)

repressed genes showed a similar rapid decrease of mRNA levels following irradiation, and remain maximally repressed 24 h after treatment. The levels of most of the newly-identified IR-induced genes rose rapidly following treatment, peaked by 4 h, and declined again to near the original levels by 24 h after treatment (data not shown), following the pattern of rapid response typical of many stress-induced immediate-early genes (Fornace, 1992; Holbrook *et al*., 1996; Smith and Fornace, 1996a). By analogy, the genes described here may have roles in acute cellular responses to damage and may share some regulatory mechanisms with previously characterized IR-response genes.

Induction of nine of the newly-identified stress-response genes was next measured in a panel of human cancer cell lines to determine the scope of their IR-response, and to monitor for induction by exposure to two DNA-base-damaging agents, the alkylating agent methyl methanesulfonate (MMS) and ultraviolet (UV) radiation. The cell lines used in this comparison included six lines of myeloid-lymphoid lineage (ML-1 (myeloid), Molt4 (lymphoid), SR (lymphoid), CCRF-CEM (lymphoid), HL60 (myeloid) and K562 (myeloid)) two lung cancer lines (A549 and H1299), two breast carcinoma lines (MCF7 and T47D) and the colon cancer line RKO along with its derivative transfected with E6 (RKO/E6) (Zhan *et al*., 1993). A summary of these results is shown in Figure 3. Induction levels of *MDM2, CIP1/WAF1* and *BAX* were also measured for comparison. These three genes are p53-regulated (Ko and Prives, 1996); in the case of *CIP1/WAF1*, it is regulated by both p53-dependent and independent mechanisms (Gorospe *et al*., 1996), while *BAX* induction by IR appears to require p53 plus an 'apoptosis-proficiency' factor frequently present in cells that undergo rapid apoptosis after IR (Zhan *et al*., 1994b). As expected, these three genes were more responsive in the p53 wt lines with little or no IR-responsiveness in the p53 deficient lines. As reported previously (Zhan *et al*., 1994b), strong *BAX* induction was only seen in myeloid-lymphoid lines with weaker induction in MCF-7 cells. In the case of the SR leukemia line, constitutive *BAX* expression was approximately an order of magnitude greater than in the other lines and

A.

	RCH1	BCL3	FRA1	REL-B	ATF3	IAP-1	PC-1	MBP-1	SSAT	MDM2	CIP1/WAF1	BAX
p53 wild type												
ML-1	0.4	5.6	6.9	57.0	9.2	2.1	19.5	3.7	3.4	12.0	31.0	9.5
Molt4	0.5	1.1	1.4	9.5	13.7	1.2	10.0	1.1	1.0	4.5	31.0	5.8
SR	0.5	0.9	1.7	5.6	6.1	2.9	3.1	2.0	1.2	8.9	8.7	1.2
A549	0.6	1.1	1.1	1.4	1.7	1.2	1.8	0.8	1.1	4.8	7.5	1.9
MCF7	0.4	1.8	9.5	4.4	1.6	1.0	0.6	1.1	1.1	2.5	22.4	2.9
RKO	0.7	2.2	0.7	3.4	5.3	3.0	3.5	1.7	1.4	2.5	4.0	1.7
p53 mutant												
CCRF-CEM	0.5	3.8	1.0	13.4	10.7	1.3	11.9	1.7	0.7	1.0	1.8	0.9
HL60	0.5	6.2	1.9	8.3	3.2	1.0	5.2	1.6	2.2	1.0	2.0	0.5
K562	0.7	1.4	1.2	1.6	0.9	1.0	1.1	1.3	1.0	1.2	1.4	1.0
H1299	0.7	0.7	0.7	2.1	1.0	1.3	4.7	1.1	0.9	1.0	1.3	1.1
RKO-E6	0.2	3.1	0.5	7.2	1.4	3.8	4.6	1.4	1.0	1.6	1.5	1.3
T47D	0.7	1.8	0	4.8	1.5	1.6	1.9	1.1	0.9	0.8	2.2	0.7

B.

		RCH1	BCL3	FRA1	REL-B	ATF3	IAP-1	PC-1	MBP-1	SSAT	MDM2	CIP1/WAF1	BAX
p53 wild type													
ML-1	MMS	0.7	0.8	0.8	1.8	7.3	0.7	1.4	0.9	1.5	9.2	11.3	2.1
Molt4	MMS	1.4	1.0	3.7	0.5	32.0	1.3	1.3	0.6	1.2	1.1	12.6	0.9
SR	MMS	1.2	0.4	1.1	1.6	10.3	1.2	1.0	0.6	1.2	3.2	6.3	0.5
A549	MMS	0.6	1.1	1.1	1.4	1.7	1.2	1.8	0.8	1.1	4.8	7.5	1.9
	UV	0.8	0.6	1.5	0.6	4.4	0.6	1.5	0.5	1.4	0.5	5.7	1.3
MCF7	MMS	0.4	1.8	9.5	4.4	1.6	1.0	0.6	1.1	1.1	2.5	22.4	2.9
	UV	0.9	1.5	24.7	1.0	3.9	0.5	1.2	1.0	1.0	2.3	9.8	1.6
RKO	MMS	0.7	2.2	0.7	3.4	5.3	3.0	3.5	1.7	1.4	2.5	6.4	1.7
	UV	0.8	1.7	0.9	0.8	4.6	1.1	1.1	1.1	1.4	1.4	2.7	1.0
p53 mutant													
CCRF-CEM	MMS	0.8	0.8	1.2	0.5	26.0	0.9	0.8	1.1	0.5	1.2	0.7	1.2
HL60	MMS	0.9	1.1	2.7	1.3	9.4	1.2	1.7	0.9	0.8	3.6	5.9	0.7
K562	MMS	1.0	1.7	1.2	0.8	37.0	0.9	0.8	0.8	1.3	1.2	1.8	0.9
H1299	MMS	0.9	0.7	0.6	1.0	21.0	0.5	1.9	0.9	0.7	0.6	6.3	0.9
	UV	0.6	0.6	0.5	1.2	3.8	0.5	3.8	0.7	0.8	0.9	2.2	1.5
RKO/E6	MMS	0.3	1.3	1.1	0.8	42.0	1.1	0.8	1.3	0.8	0.9	2.3	0.7
	UV	0.2	1.0	0.7	0.7	2.8	0.5	0.6	0.7	0.9	0.5	2.8	1.0
T47D	MMS	0.8	0.8	0	1.6	38.0	1.5	0.7	0.8	0.9	0.7	3.9	0.8
	UV	1.1	1.3	0	1.9	3.5	1.5	3.3	0.5	1.2	0.9	2.7	0.8

Figure 3 Regulation of transcripts identified on the microarray measured in a panel of human cancer cell lines. Numbers shown are the relative induction for each gene over levels in untreated controls 4 h after treatment with (**a**) 20 Gy ionizing radiation or (**b**) 100 μg/ml methyl methanesulfonate (MMS) or 14 J/m^2 ultraviolet (UV) radiation as measured by quantitative dot-blot hybridization (Hollander and Fornace, 1990). A zero indicates no detectable expression in either control or treated cells. The results are color coded: red for >twofold induction, green for >twofold reduction, and yellow for <twofold change from untreated control

appreciable induction was not seen. In contrast to these previously described genes, the ionizing radiation response of the newly characterized genes shows far more heterogeneity among different cell lines (Figure 3a). While all of the newly-identified genes respond to IR in at least one cell line in addition to ML-1, two of the cell lines, K562 and A549, did not show γ-ray regulation of any of the newly-defined IR-responsive genes, and only *ATF3* was induced by any stress agent tested in these two lines (Figure 3b).

Interestingly, K562 was the least responsive of any of the cell lines, and was the only line derived from a patient with chronic myelogenous leukemia. Of the nine genes screened, 5(SSAT, c-Myc Promoter binding protein (*MBP-1, or PRDII-BF1*) (Fan and Maniatis, 1990), cellular inhibitor of apoptosis 1 (*c-IAP1*)

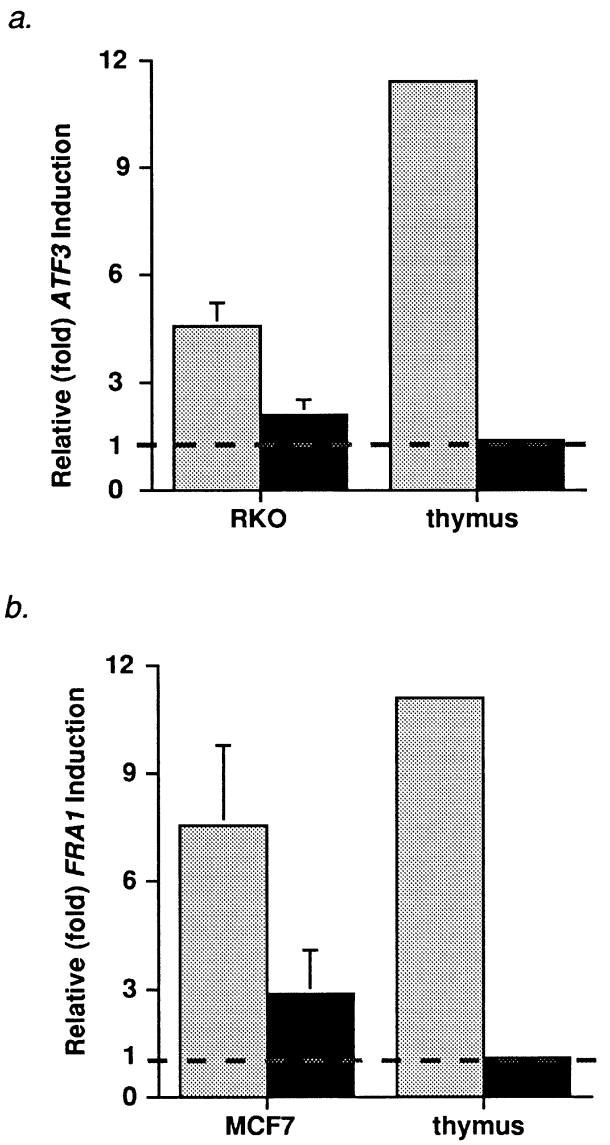

Figure 4 (**a**) Induction over basal levels of *ATF3* 4 h after 20 Gy IR in p53 wild-type RKO cells (light bar), or in RKO/E6 (dark bar), in which p53 function has been abrogated by an E6 expression vector, and 2 h after IR in the thymus of p53 wild-type mouse (light bar), or p53−/− (dark bar). The results shown for the RKO cell lines are the average of four independent experiments, and error bars are standard errors of the means. The dotted line marks the basal level corresponding to a relative induction of 1.0. (**b**) Induction over basal levels of FRA-1 in p53 wild-type MCF7 cells (light bar), or in MCF7/E6 (dark bar) in which p53 function has been abrogated by an E6 expression vector, and in the thymus of p53 wild-type mouse (light bar), or p53−/− mouse (dark bar). The results for the MCF7 cell lines are the average of three independent experiments

(Rothe *et al*., 1995), *RELB* (Liou *et al*., 1994) and *BCL3* (Zhang *et al*., 1994)) were primarily induced by IR in the 12 cell lines examined. Fos-related antigen-1 (*FRA-1*) (Cohen and Curran, 1988), RCH1, and prohormone convertase1 (*PC1*) or neuroendocrine convertase1 (*NEC1*) (Seidah *et al*., 1991), all showed regulation by the base-damaging agents MMS or UV radiation in some cell lines (Figure 3b). The most wide ranging response was seen for activating transcription factor 3 (*AFT3*) (Liang *et al*., 1996), which was induced by both MMS and UV radiation in all cell lines tested. With such a strong and pervasive response, ATF3 is likely to play an important role in generalized genotoxic stress responses. The complex patterns seen in Figure 3 emphasize the importance of cellular context in genotoxic stress responses and highlight the need for high throughput techniques for the analysis of these responses, since even with only 12 targets, each stress and cell line had a unique pattern of induction.

The induction patterns in Figure 3 may hold clues to regulation of the induction of some of these genes. For instance, the effect of the p53 status of a cell on its ability to induce *CIP1/WAF1* (El-Deiry *et al*., 1993; Zhan *et al*., 1995) and *MDM2* (Chen *et al*., 1994b; Perry *et al*., 1993) in response to IR has been well documented. This effect is reflected in the weak to absent IR-induction of these two transcripts in p53 mutant cell lines in this panel compared to the clear IR-induction in all p53 wild type lines examined. Although not so widely induced, *FRA-1* showed a similar pattern in that it was not induced by IR in any of the p53 mutant lines in this panel. In addition, the fivefold IR-induction of *ATF3* in RKO was attenuated in the RKO/E6 cell line, although some IR-induction was seen among the other p53 mutant cell lines. These results raised the possibility that the IR-induction of *FRA-1* and *ATF3* may involve a p53 regulatory component. Figure 4a shows the IR-induction of *ATF3* in the p53 wild-type human carcinoma cell line RKO and in RKO/E6, in which p53 function has been abrogated by an E6 expression vector. Both these lines have been well characterized in our laboratory (Smith *et al*., 1995; Zhan *et al*., 1993, 1994a), and RKO/E6 has been shown to lack appreciable functional p53. The disruption by E6 of *ATF3* induction by IR supports a role for p53 in its induction. To further test the extent of dependence of *ATF3* IR-induction on p53 status, we examined the *in vivo* induction in wild-type and p53−/− (knockout) mice (Donehower *et al*., 1992) using 5 Gy whole-body γ-irradiation. While *ATF3* was well induced by 2 h after irradiation in the thymus of wild-type mice, there was no significant induction in the p53−/− mouse (Figure 4a). *ATF3* levels remained elevated at 4 and 8 h after irradiation in the thymus of wild-type mice, without any induction in the p53−/−. A similar trend was seen in the liver, where *ATF3* was induced about fourfold in the wild-type mouse, and not induced in the p53 knockout (data not shown).

ATF3 is a member of the activating transcription factor/cAMP response element binding protein (ATF/CREB) family which homodimerizes to repress transcription from promoters with ATF sites. An alternatively spliced form of the *ATF3* transcript, which lacks DNA binding activity, is also expressed in cells, but this form promotes transcription (Chen *et al*., 1994a). Based on the sizes of *ATF3* transcripts hybridizing on the Northern blot, the smaller alternatively spliced form was the major transcript expressed in untreated ML-1 cells, whereas the IR-induced transcript was predominantly of the full length form (Figure 2). Full length ATF3 can also form a heterodimer with the stress-protein Gadd153, preventing its usual transcriptional repression (Chen *et al*., 1996). ATF3/Jun heterodimers, on the other hand, activate transcription in transient transfection assays (Hsu *et al*., 1992). Adenovirus E1A (AdE1A) expression also induces *ATF3*, and leads to the formation of

cJun/ATF3 heterodimers, possibly contributing to oncogenic transformation by AdE1A (Hagmeyer *et al*., 1996). ATF3 has previously been shown to be induced by serum stimulation, 12-*O*-tetradecanoylphorbol-13-acetate (Chen *et al*., 1994a), and by physiological stresses such as wounding, $CC1_4$ and alcohol intoxication, ischemia/reperfusion and brain seizure (Chen *et al*., 1996). The induction of *ATF3* by the DNA-damaging agents MMS and UV radiation in all 12 cell lines tested in this study extends the range of stress responses in which ATF3 is involved. Its wide-ranging responsiveness is reminiscent of *CIP1/WAF1* and *GADD45* which are stress inducible by both p53-dependent and independent mechanisms (Holbrook *et al*., 1996; Zhan *et al*., 1995), and highlights the complexity of *ATF3* regulation.

Although *FRA-1* was not IR-inducible in RKO cells, a similar comparison was possible using the MCF7 and MCF7/E6 cell lines. The human breast carcinoma cell line MCF7 has wild-type p53, while MCF7/E6 has been demonstrated to lack appreciable wild-type p53 function (Smith *et al*., 1995; Zhan *et al*., 1993, 1994a), and both cell lines have been well characterized in our laboratory. The reduced IR-induction of *FRA-1* in MCF7/E6 compared to MCF7 again supports a role for p53 in the IR-induction of this gene (Figure 4b). Furthermore, whole-body γ-irradiation of wild-type mice resulted in *FRA-1* induction in the thymus, but no induction occurred in the thymus of the p53−/− (Figure 4b). FRA-1 is an immediate early gene induced by serum stimulation, the product of which shares several regions of amino acid homology with Fos (Cohen and Curran, 1988). It has also been reported to be down-regulated by UVB and upregulated by UVA (Ariizumi *et al*., 1996). Because of its homology to Fos, and the involvement of p53 in its IR-induction, *FRA-1* may represent a link between p53 and AP1 function and the MAPK pathway.

MBP-1 represented another potentially p53 regulated gene, showing a pattern similar to that seen for *BAX* or *BCL-X*, in that it was induced only in p53-wild-type cell lines of lymphoid or myeloid lineage. We examined the induction of the murine homolog of *MBP-1* in the tissues of wild-type and p53−/− mice, and found marginal to absent expression in liver and thymus, but strong expression in spleen. Treatment with ionizing radiation resulted in a twofold induction of this gene in the spleens of both p53 wild-type and p53−/− mice, suggesting that this gene does not require p53 function for its induction, but that its expression and induction are both limited to a subset of cell types. This would be consistent with a role for *MBP-1* in tissue specific p53-independent stress responses.

The high variability of transcriptional responses found in different cell lines (Figure 3) emphasizes that a single cell line or cell type cannot provide a general model for cellular responses to genotoxic stress. The further finding that only two of nine of these genes examined in this cell line panel showed a recognizable p53 component to their regulation belies the recent focus of stress-gene studies primarily on p53-regulated genes. For instance, another study, submitted shortly after this one, detected IR-regulation of multiple transcripts in human fibroblasts engineered to express high levels of cloned p53, but induction of nearly all of these genes was p53-dependent (Komarova *et al*., 1998). Interestingly, when this study was extended to tissues of mice irradiated *in vivo*, the majority of regulated genes found were responsive in a tissue-specific manner, again underlining the importance of cellular context to stress-gene response. Other gene discovery approaches (Madden *et al*., 1997; Polyak *et al*., 1997) have also focused on p53-dependent gene induction. Exclusive focus on such approaches using engineered cell lines would likely overlook many IR-response genes, such as the majority of those described here in myeloid cells. In light of the loss of functional p53 in the majority of tumors, the non-p53-dependent stress response genes may be an important consideration in cancer treatment.

Cellular context is clearly critical in determining which genes can be induced, and stress responses probably involve multiple signaling pathways (Holbrook *et al*., 1996; Zhan *et al*., 1996). It is likely that the patterns of transcriptional responses beginning to emerge in Figure 3 are indicative of as yet undiscovered cell-type-specific regulatory factors or the interruption of signal transduction pathways in some cancer cell lines. Signaling pathways involving p53, NFκB, the MAP kinases, and others have roles in both protective and apoptotic responses after genotoxic stress (Holbrook *et al*., 1996; Smith and Fornace, 1996b), and many of the genes responding on this microarray interact with members of these pathways. For instance, *RELB*, a modifier of NFκB subunit binding affinity was induced in 10/12 cell lines studied, but only by IR. As NFκB activation is a fairly universal response to genotoxic stress, transcriptional regulation of this subunit may be one mechanism by which this activity is channeled into a more specific downstream response. By changing the balance of specific NFκB heterodimers in the nucleus, an increase in RELB changes the affinity of NFκB for promoter binding, resulting in differential regulation of some NFκB targets (Ivanov *et al*., 1995; Liou *et al*., 1994). If an increase in *RELB* mRNA levels does occur only in response to an IR-specific signal or damage, this could represent an important discriminator of stress-response.

In light of the complexity of cellular transcriptional stress response highlighted in this study, quantitative functional genomics approaches, such as cDNA microarray hybridization, will be needed to unravel the inter-relationships of the molecular response pathways involved. Although radioactive-probe hybridization to nylon filter arrays provides a useful method to screen for potential genes of interest which differ in expression levels between two samples, differential screening has its own limitations (Fargnoli *et al*., 1990); some of which are avoided by the use of probes labeled with different fluorochromes co-hybridized to the same microarray. Other methods for identification of differentially expressed mRNAs, such as differential display, subtractive library hybridization and serial analysis of gene expression (SAGE) (Velculescu *et al*., 1995), can be biased toward detection of highly-expressed and/or strongly-induced transcripts. With the microarray approach reported here, quantitative results over a wide dynamic range were obtained for many genes. The application and further refinement of quantitative fluorescent cDNA microarray hybridiza-

tion have the potential to advance our understanding of the fields of stress gene response and radiation biology, and to extend this technology beyond simple pair-wise comparisons to applications such as tumor typing, pharmacological screening, biomonitoring, and rapid carcinogen screening.

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References

Ariizumi K, Bergstresser PR and Takashima A. (1996). *J. Dermatol. Sci.,* **12,** 147–155.
Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G and Thompson CB. (1993). *Cell,* **74,** 597–608.
Chen BP, Liang G, Whelan J and Hai T. (1994a). *J. Biol. Chem.,* **269,** 15819–15826.
Chen BP, Wolfgang CD and Hai T. (1996). *Mol. Cell. Biol.,* **16,** 1157–1168.
Chen CY, Oliner JD, Zhan Q, Fornace Jr AJ, Vogelstein B and Kastan MB. (1994b). *Proc. Natl. Acad. Sci. USA,* **91,** 2684–2688.
Chen Y, Dougherty ER and Bittner ML. (1997). *J. Biomed. Optics,* **2,** 364–374.
Chittenden T, Harrington EA, O'Connor R, Flemington C, Lutz RJ, Evan GI and Guild BC. (1995). *Nature,* **374,** 733–736.
Cohen DR and Curran T. (1988). *Mol. Cell. Biol.,* **8,** 2063–2069.
Cuomo CA, Kirch SA, Gyuris J, Brent R and Oettinger MA. (1994). *Proc. Natl. Acad. Sci. USA,* **91,** 6156–6160.
DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA and Trent JM. (1996). *Nat. Genet.,* **14,** 457–460.
Donehower LA, Harvey M, Slagle BL, McArther MJ, Montgomery Jr CA, Butel JS and Bradley A. (1992). *Nature,* **356,** 215–221.
El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B. (1993). *Cell,* **75,** 817–825.
Fan CM and Maniatis T. (1990). *Genes Dev.,* **4,** 29–42.
Fargnoli J, Holbrook NJ and Fornace Jr AJ. (1990). *Anal. Biochem.,* **187,** 364–373.
Fornace Jr AJ. (1992). *Ann. Rev. Genetics,* **26,** 507–526.
Gorospe M, Martindale JL, Sheikh MS, Fornace Jr AL and Holbrook NJ. (1996). *Mol. Cell. Differentiation,* **4,** 47–65.
Hagmeyer BM, Duyndam MC, Angel P, de Groot RP, Verlaan M, Elfferich P, van der Eb A and Zantema A. (1996). *Oncogene,* **12,** 1025–1032.
Heller RA, Schena M, Chai A, Shalon D, Bedilion T, Gilmore J, Woolley DE and Davis RW. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 2150–2155.
Holbrook NJ, Liu Y and Fornace Jr AJ. (1996). In: *Stress-inducible Cellular Responses*. Feige U, Morimoto RI, Yahara I and Polla BS. (eds). Birkhauser Verlag: Basel, Switzerland. pp. 273–288.
Hollander MC and Fornace Jr AJ. (1990). *Biotechniques,* **9,** 174–179.
Hsu JC, Bravo R and Taub R. (1992). *Mol. Cell. Biol.,* **12,** 4654–4665.
Ivanov VN, Deng G, Podack ER and Malek TR. (1995). *Int. Immunol.,* **7,** 1709–1720.
Ko LJ and Prives C. (1996). *Genes Dev.,* **10,** 1054–1072.
Komarova EA, Diatchenko L, Rokhlin OW, Hill JE, Wang ZJ, Krivokrysenko VI, Feinstein E and Gudkov AV. (1998). *Oncogene,* **17,** 1089–1096.
Lashkari DA, DeRisi JL, McCusker JH, Namath AF, Gentile C, Hwang SY, Brown PO and Davis RW. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 13057–13062.
Liang G, Wolfgang CD, Chen BP, Chen TH and Hai T. (1996). *J. Biol. Chem.,* **271,** 1695–1701.
Liou HC, Sha WC, Scott ML and Baltimore D. (1994). *Mol. Cell. Biol.,* **14,** 5349–5359.
Madden SL, Galella EA, Zhu J, Bertelsen AH and Beaudry GA. (1997). *Oncogene,* **15,** 1079–1085.
Perry ME, Piette J, Zawadzki JA, Harvey D and Levine AJ. (1993). *Proc. Natl. Acad. Sci. USA,* **90,** 11623–11627.
Polyak K, Xia Y, Zweler JL, Kinzler KW and Vogelstein B. (1997). *Nature,* **389,** 300–306.
Rothe M, Pan MG, Henzel WJ, Ayres TM and Goeddel DV. (1995). *Cell,* **83,** 1243–1252.
Schena M, Shalon D, Davis RW and Brown PO. (1995). *Science,* **270,** 467–470.
Schena M, Shalon D, Heller R, Chai A, Brown PO and Davis RW. (1996). *Proc. Natl. Acad. Sci. USA,* **93,** 10614–10619.
Seidah NG, Mattei MG, Gaspar L, Benjannet S, Mbikay M and Chretien M. (1991). *Genomics,* **11,** 103–107.
Smith ML, Chen IT, Zhan Q, O'Connor PM and Fornace Jr AJ. (1995). *Oncogene,* **10,** 1053–1059.
Smith ML and Fornace Jr AJ. (1996a). *Mutation Res.,* **340,** 109–124.
Smith ML and Fornace Jr AJ. (1996b). *Am. J. Pathol.,* **148,** 1019–1022.
Velculescu VE, Zhang L, Vogelstein B and Kinzler KW. (1995). *Science,* **270,** 484–487.
Zhan Q, Carrier F and Fornace Jr AJ. (1993). *Mol. Cell. Biol.,* **13,** 4242–4250.
Zhan Q, Bae I, Kastan MB and Fornace Jr AJ. (1994a). *Cancer Res.,* **54,** 2755–2760.
Zhan Q, Fan S, Bae I, Guillouf C, Liebermann DA, O'Connor PM and Fornace Jr AJ. (1994b). *Oncogene,* **9,** 3743–3751.
Zhan Q, El-Deiry W, Bae I, Alamo Jr I, Kastan MB, Vogelstein B and Fornace Jr AJ. (1995). *Int. J. Oncology,* **6,** 937–946.
Zhan Q, Alamo Jr I, Yu K, Boise LH, O'Connor PM and Fornace Jr AJ. (1996). *Oncogene,* **13,** 2287–2293.
Zhan Q, Bae I, Fornace Jr AJ and Craig RW. (1997). *Oncogene,* **14,** 1031–1039.
Zhang Q, Didonato JA, Karin M and McKeithan TW. (1994). *Mol. Cell. Biol.,* **14,** 3915–3926.